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## **Daphniid zooplankton assemblage shifts in response to eutrophication and metal contamination during the Anthropocene**

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## PROCEEDINGS B

**Daphniid zooplankton assemblage shifts in response to eutrophication and metal contamination during the Anthropocene**

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**Title:** Daphniid zooplankton assemblage shifts in response to eutrophication and metal contamination during the Anthropocene

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**Abstract:** Human activities during the Anthropocene result in habitat degradation that has been associated with biodiversity loss and taxonomic homogenisation of ecological communities. Here we estimated effects of eutrophication and heavy metal contamination, separately and in combination, in explaining zooplankton species composition during the past 125-145 years using analysis of daphniid diapausing egg banks from four lakes in the Northeastern USA. We then examined how these community shifts influenced patterns of diversity and homogenisation. Analysis of past lake production (via subfossil pigments) and metal contamination (via sedimentary metals) demonstrated that eutrophication alone (19-39%) and in combination with metal pollution (17-54%) explained 36-79% of historical variation in daphniid species relative abundances in heavily fertilised lakes. In contrast, metal pollution alone explained the majority (72%) of historical variation in daphniid assemblages at the oligotrophic site. Several species colonisation events in eutrophying lakes resulted in increased species richness and gamma diversity through time. At the same time, daphniid assemblages in three eutrophied lakes became more similar to each other (homogenised) but this pattern was only seen when accounting for species presence/absence. We did not observe consistent patterns of divergence between the assemblages in the eutrophying lakes and the low-nutrient reference site. Given the pervasive nature of fertilisation and metal pollution and the sensitivity of cladocerans to these factors, we suggest that many inhabited lake districts may already exhibit similar patterns of daphniid assemblage shifts.

**Key words:** biotic homogenisation, Jaccard similarity, eutrophication, *Daphnia*, heavy metals, palaeolimnology

# **Introduction**

We have entered the Anthropocene, an era during which human activities can profoundly influence the Earth’s geological, chemical, and biological processes at a global scale [1]. One pervasive signature of land use change in this human dominated era is the increasing release and accumulation of chemical pollution in the environment. At the same time, biodiversity loss has been precipitous, and this has long been cited as a consequence of widespread anthropogenic environmental changes, including chemical pollution [2,3]. However, it can be difficult to causally link pollution with changes in species diversity. In addition, increasing biotic homogenisation of communities in human impacted environments is an emerging pattern gaining increasing recognition [4,5]. As developed landscapes begin to predominate on Earth, sensitive endemic species are often lost while human-tolerant species spread, a pattern that can reduce biotic diversity at regional scales. Such assemblage shifts have been observed in a number of groups and habitat types around the world including plants [6], molluscs [7], fish [8], amphibians and reptiles [9]. One hypothesis is that habitat degradation associated with agricultural and urban activity homogenises the physical landscape and thus its constituent ecological communities [10,11].

Human activities routinely change nutrient influx to lakes and can have transformative impacts on aquatic ecosystems [12]. Elevated nutrient input directly increases phytoplankton productivity and can favour blooms of toxic cyanobacteria [13], while increased phytoplankton biomass indirectly alters aquatic ecosystems by creating deep-water anoxia which can cause extensive fish kills [14] and phosphorus (P) release from sediments [12]. While eutrophication may proceed steadily, the ecological response of higher trophic level taxa such as invertebrates and fish can be complex, non-linear, or even threshold-like [15–17]. These long-term effects of

61 nutrient accumulation on lake communities have been well documented (e.g., [18–20]), but are  
62 typically studied in isolation from other stressors such as heavy metals (but see [21]). Yet,  
63 nutrients and other chemical contaminants of freshwaters occur together, thus it is important to  
64 understand their joint impacts on ecological communities.

65 Industrial development has increased the release of heavy metals (e.g., mercury [Hg],  
66 cadmium [Cd], lead [Pb], chromium [Cr], copper [Cu], zinc [Zn]) into the environment [22]  
67 where they accumulate in lake basins, even in remote locations (e.g., [23–25]). Moreover, Cu has  
68 been widely applied as an algicide in eutrophic lakes [26]. While heavy metals occur naturally,  
69 at elevated concentrations most heavy metals, even essential elements such as Zn [27], are toxic  
70 to living organisms. Toxicological testing has generated a large body of information about short-  
71 term effects of individual metals on organisms in controlled laboratory environments (e.g. US  
72 EPA’s ECOTOX database: <https://cfpub.epa.gov/ecotox>). Yet how effects of heavy metals play  
73 out over multi-decadal time scales in field conditions remains uncertain and represents an  
74 important research frontier for ecotoxicology [28].

75 Freshwater zooplankton are often sensitive to alterations of the chemical environment  
76 and offer an excellent model system to study the unique and interactive effects of eutrophication  
77 and metal pollution. In particular, *Daphnia* species are keystone herbivores in lake food webs  
78 [29] that respond strongly to eutrophication because of changes in resource availability [30],  
79 phytoplankton size structure and grazing sensitivity [15], and exposure to cyanobacterial toxins  
80 [31], among other factors. Daphniids are also sensitive to heavy metal exposure, although  
81 susceptibility to individual elements varies among species [32]. Despite these observations,  
82 relatively little is known of how planktonic invertebrate community composition has varied with

anthropogenic forcing over a century of eutrophication and metal contamination in much of the industrialised world (but see [21,33]).

In this study, we use lake sediment archives to examine long-term (ca. 145 years) ecological responses of daphniid zooplankton assemblages to nutrient fertilisation and heavy metal contamination. Both stressors are common consequences of anthropogenic land use, are known to affect zooplankton, and leave unique signatures in lake sediments, allowing their effects on lake biota to be studied over long time spans [34,35]. We estimated the relative influence of these pollutants on temporal patterns of daphniid species composition in these lakes and determined whether the effects of these contaminants resulted in the loss of diversity or assemblage shifts seen in other taxonomic groups in human dominated landscapes.

**Materials and methods**

*Lake selection:* Four study lakes in Connecticut, USA, were chosen representing a wide range of nutrient influx over the past eighty years [36,37] (electronic supplementary material (ESM) 1: table S1). Based on historic total phosphorus (TP) records, Black Pond has remained oligotrophic and serves as a low-nutrient reference site, whereas Alexander Lake has become mesotrophic, in part due to P supply from sediments. Cedar Pond and Roseland Lake have both become hypereutrophic and are listed as impaired for recreation (both lakes) and the support of aquatic life (Cedar) by the United States Environmental Protection Agency. Typical of lakes in the region (ESM1: table S2), all study lakes are relatively small and shallow, but vary in physical and chemical characteristics as well as the degree of historical changes in land use within their watersheds [38] (ESM1: text S1, table S1). We restricted our study to lakes without landlocked

alewives (*Alosa pseudoharengus*) to avoid confounding influences of change in trophic regimes arising from management of zooplanktivorous fish stock [39,40] (ESM1: text S2).

*Sediment collection and dating:* Methods for sediment collection, sectioning, storage, and establishment of geochronology are described in detail elsewhere [38]. Briefly, 12.5-cm diameter sediment cores were collected from the deepest basin of each lake by SCUBA divers. Cores were sectioned at 1.5-cm intervals and stored in the dark at 4°C for examination of daphniid diapausing egg densities. Sediments for examination of photosynthetic pigments and nutrients were frozen. Rogalski [38] estimated sediment ages for these study lakes based on changes in  $^{210}\text{Pb}$  radioisotope activity with depth (ESM2: figure S1).

*Heavy metal analysis:* Sediment concentrations of Cd, Cr, Cu, Pb, and Zn were measured by inductively coupled plasma-mass spectrometry (Thermo Finnigan Element 2 high resolution ICP-MS) following extraction by hot block acid digestion. Sediment Hg concentrations were measured by atomic absorption spectrometry (Direct Mercury Analyzer DMA80). Additional methodological details are published elsewhere [38].

*Nutrient and subfossil pigment analyses:* Total carbon and total nitrogen composition of sediments were measured on whole freeze-dried sediments. Samples of 2-10 mg dry mass were packed into tin capsules and introduced into a NC-2500 elemental analyser. Nitrogen and C components of sediments were oxidised completely at 1000°C in a furnace to convert organic constituents into simple nitrogen-based gases and CO<sub>2</sub>. Elemental mass ratios were estimated using combustion data. Sedimentary pigments were extracted, filtered, and dried under N<sub>2</sub> gas following the standard procedures of Leavitt and Hodgson [35] (ESM2: text S3).

*Daphniid community structure:* Historical changes in species composition were quantified using sedimentary banks of diapausing eggs for taxa in the Daphniidae family [41], a



group of filter-feeding crustacean zooplankton that are keystone herbivores in lakes [29] and preserve well in lake sediments [20,41]. Diapausing eggs (encased in ephippia) were isolated by filtering whole sediments through 50- $\mu$ m mesh and examining the retained filtrate under a dissecting microscope at 10 $\times$  power. All ephippia were examined with a compound microscope at 100 $\times$  and 400 $\times$  power to compare gross morphology and finer details of specimens. In most cases, ephippia were identified from sediment subsamples until at least 50 specimens (median 73) per sediment slice were enumerated, beginning with surface sediment and examining alternate sediment sections over the past 125-145 years. For the earliest time period from Black and Alexander lakes, only 42 and 46 ephippia were available for counting, respectively.

We identified ephippia to species by hatching animals from a subset of viable eggs representing all morphological types found in the four lakes. Intact ephippia were incubated in COMBO freshwater medium [42] using spring light and temperature conditions (14:10h light: dark, 15°C). Hatchlings were cultured in COMBO medium and fed *Scenedesmus obliquus* until they reached maturity and could be identified to species. We then matched daphniid species identity to the six ephippial morphotypes based on characteristics of the ephippia from which the hatchlings originated (ESM3: text S4). Historical species densities in subfossil daphniid assemblages were based on combined counts of empty and intact ephippia present in the sediments, unless ephippia were crushed or too decomposed to be identified (1.1% of counts).

*Relative importance of eutrophication and metals:* We used multivariate statistical analyses to quantify the relative importance of heavy metal contamination and eutrophication in explaining variation in daphniid species composition through time. Owing to the large number of variables describing changes in metal contamination and eutrophication, principle components analysis (PCA) was conducted on data from each lake to reduce each type of pollution to a

univariate index (ESM4: table S3). We used PC1 axis loading scores of metals and of eutrophication as explanatory variables in a redundancy analysis (RDA) predicting daphniid zooplankton assemblage structure (Hellinger-transformed species abundances). In Cedar Pond, temporal patterns of Cu contamination greatly differed from the other metals and were not reflected in the metals PC1. In this case, Cu was excluded from the metals PCA and was included as a separate predictor, in addition to the eutrophication and metals PC1 scores. Following Borcard et al. [43] and using adjusted  $R^2$ , we used variance partitioning to determine the amount of variance in community structure explained by metals, eutrophication, and a combination of these stressors [44]. Monte Carlo permutation tests (10,000 permutations) were used to test the significance of these associations. RDA and variance partitioning were conducted separately for each lake using data from 6-9 time periods per lake.

*Taxonomic similarity analysis:* Studies of taxonomic homogenisation typically compare community similarity between two time intervals to gauge whether the communities have become more similar over time [45]. We used this approach to compare overall changes in daphniid community composition among the four study lakes since ca. 1860, a period of significant change in regional metal contamination and eutrophication [23,38,46]. We recognize that estimates of historical change in the relative abundance of daphniids in an egg bank reflect both variation in the past density of animals in the water column as well as their tendency to produce ephippia through sexual reproduction, a factor which can vary through time and among species [47]. We estimated overall changes in community similarity using the Jaccard similarity index ( $J$ ). This measure based on species presence and absence is commonly used in studies of biotic homogenisation [48] and allowed us to characterise community changes related to the colonisation or local extinction of species. Similarity values range from 0 to 1, with 1

representing complete overlap in species composition and 0 representing no shared species between two sites. We tested whether daphniid communities became more taxonomically homogeneous over time among pollution-impacted lakes by subtracting historic baseline  $J$  values (ca. 125-145 years BP) from modern (surface sediment) values. We also assessed whether the communities in the heavily eutrophied lakes diverged from the reference community in Black Pond over time.

Our data set also enabled a more detailed examination of temporal shifts in daphniid composition in the four lakes since ca. 1860. We conducted PCA using Hellinger transformed daphniid species densities in all four lakes. PCA biplots allowed us to visualize the timing and nature of any patterns of convergence and divergence in community composition among the four study lakes. In addition, we calculated the Euclidean distance between historic and modern daphniid assemblages in each lake, using the first three PC axis scores (based on a scaling that preserves Euclidean distances in multidimensional space). We subtracted modern from historic PCA distances, such that a positive difference indicated an increase in similarity between assemblages over time. We conducted PCA, RDA, and variance partitioning using the package *vegan* [49] with the statistical software R version 3.0.2 [50].

*Species richness trends:* We made rarefied estimates of species richness at each time period based on random subsamples of 42 ephippia (smallest sample size of any of the lakes/ time periods). This analysis was conducted using the rarefy function in the R package *vegan*.

## Results

*Heavy metal contamination:* The timing and magnitude of metal contamination differed among lakes and metals [38] (figure 1, ESM4: figure S2). Metal contamination typically increased

beginning in the early 1900s and either levelled off or continued to rise over time. The exception is Cedar Pond, where metals peaked earlier and declined in recent decades. The largest changes occurred in Cu, Cd, and Hg, metals known to be highly toxic to cladoceran zooplankton [51,52]. Concentrations of Cu increased rapidly between the 1940s-90s and remained elevated in Roseland Lake owing to consistent annual application of hundreds of kg of copper sulphate ( $\text{CuSO}_4$ ) to manage nuisance phytoplankton blooms (personal communication, Putnam County, CT Water Department). Based on changes in sediment Cu contamination, we believe this practise also occurred in Cedar Pond but ceased after the 1980s, although documentation of practises is unavailable. Cd and Hg contamination likely entered the lakes through regional atmospheric deposition resulting from industrial activity, including fossil fuel burning [22,53]. Overall, sediment concentrations of copper in Roseland Lake (ca. 1980s-modern sediments), copper in Cedar Pond (at its peak in ca. 1982) and lead in Cedar Pond (ca. 1916-1969) reached levels above probable effect concentrations (i.e. harmful effects to aquatic life are considered likely; ESM4: figure S2) [54].

*Eutrophication:* The degree and timing of anthropogenic eutrophication also varied among lakes and roughly followed trajectories expected from regional monitoring programs (figure 1, table S1, ESM4: figure S3). Analysis of multiple pigment and nutrient biomarkers suggests that eutrophication was minimal in Black Pond, moderate in Roseland Lake, and substantial in Alexander Lake and Cedar Pond. Eutrophication proceeded steadily over the past century in Cedar Pond and Roseland Lake and was more pronounced over the past 50 years in Alexander Lake (figures 1, S3). In all cases, sediment C: N ratio declined to some extent during the past 100 years, consistent with increased deposition of phytoplankton biomass [55]. Subfossil pigment concentrations were relatively unchanged during the past 125-145 years in Black Pond

(23% increase in total pigments), increased moderately in Roseland Lake (172% increase), and rose substantially in Alexander Lake and Cedar Pond (334% and 405%, respectively) (figure S3). Increases in the pigment okenone, produced by obligately anaerobic photosynthetic purple sulphur bacteria, indicate that anoxia extended into the photic zone with increasing regularity in Cedar Pond (after 1900) and Alexander Lake (after 1950). The relative concentration of subfossil pigments produced by cyanobacteria (myxoxanthophyll, canthaxanthin, echinenone) increased in Cedar and secondarily Roseland, but remained unchanged in Alexander Lake and Black Pond (ESM4: figure S3).

*Daphniid community composition:* Five *Daphnia* (*D. ambigua*, *D. catawba*, *D. mendotae*, *D. parvula* and *D. pulicaria*) and one *Ceriodaphnia* species were identified in the four lakes. *Ceriodaphnia ephippia* were rarely viable and were therefore identified to the genus level. *D. mendotae* and *D. pulicaria* colonised Cedar Pond by the 1930s, while *D. mendotae* colonised Roseland and Alexander Lakes by the 1970s and 1990s respectively (figure 1). *D. pulicaria* was absent from the sediment record in Cedar Pond beginning in the 1960s and was seen again in the sediments ca. 2004-2011. *D. pulicaria* was present in low abundances from the beginning of the sediment record in Alexander Lake. Overall, large-bodied *D. pulicaria* and *D. mendotae* were rare (1-5% relative abundance) for as long as 80 years before rapidly increasing in abundance over the past 10-30 years. Aside from these colonisation and population expansion events, daphniid assemblages remained remarkably consistent in species relative abundances, particularly prior to 1950 (figure 1). Local extinction occurred in only one instance (*Ceriodaphnia* in Roseland, which comprised <1% of the community in 1904). *Ceriodaphnia* was generally uncommon when present and declined in abundance in all lakes.

*Influence of environmental change on community structure:* Variance partitioning with redundancy analysis revealed that the abundance of daphniid species in nutrient-polluted lakes was strongly associated with variation in nutrients, either alone or in concert with metal pollution. Eutrophication alone explained 18.7-39.1% of historical community variation, while the combined (statistically-inseparable) effects of eutrophication and metals explained an additional 17.3-53.6% of the variation (table 1). In contrast, variation in the daphniid assemblage in oligotrophic Black Pond was associated strongly with changes in metal contamination alone (adj.  $R^2 = 72\%$ ). Copper was a significant predictor ( $p=0.047$ ,  $F=2.92$ ) of daphniid species abundances in Cedar Pond in an RDA model that included eutrophication (PC1). Variation partitioning showed that copper explained 14.8% (adj.  $R^2$ ) of the variation in the daphniid assemblage in Cedar that was not explained by eutrophication (table 1). RDA biplots indicate that two species, *D. mendotae* and *D. pulicaria*, were associated with states of high phytoplankton abundance (figure 2b-d), while both species were absent from unproductive Black Pond (figure 1). *D. pulicaria* was also associated with low copper time periods in Cedar Pond (figure 2c). *Ceriodaphnia* was characteristic of low metal and low nutrient regimes (figure 2).

*Community similarity among lakes:* The daphniid assemblages in strongly eutrophying lakes (i.e., Alexander, Roseland, and Cedar) became more similar to one another over the past 125-145 years, as evidenced by a positive change in  $J$  over this time interval (figure 3a). Community comparisons between the eutrophying lakes and the low-nutrient reference community in Black Pond were less consistent. The daphniid assemblages in Cedar Pond and Alexander Lake diverged from that in Black Pond (figure 3b), while the daphniid assemblage in eutrophied Roseland Lake became more similar to that in Black Pond.

PCA showed that over the past century the assemblages in Alexander Lake, Cedar Pond, and Roseland Lake followed similar trajectories along PC axis 3 (figure 4, ESM4: table S4), a pattern which reflects the colonisation and increase of *D. pulicaria* and *D. mendotae* in these eutrophying lakes through time. The assemblage in the low nutrient site Black Pond did not change along the PC3 axis, as neither of these species were ever present in the sediment record. At the same time, the assemblages in Alexander Lake, Cedar Pond and Black Pond shifted towards the left along PC axis 1, indicating an increase in *D. catawba* and a decrease in *Ceriodaphnia* and *D. ambigua*. The assemblage in Roseland shifted in the opposite direction along the PC1 axis. Additional information on daphniid assemblage shifts along PC axes 1-2 and 2-3 are provided in figure S4 (ESM4).

Euclidean distances calculated using the first three PC axis scores showed that overall the Alexander and Roseland daphniid assemblages became more similar while diverging from the assemblage in Cedar Pond (Figure 3a). The assemblages in Alexander Lake and Cedar Pond diverged from the assemblage in the low nutrient site, Black Pond (figure 3b), while the assemblage in eutrophying Roseland Lake became more similar to that in Black Pond.

*Species richness:* The low-nutrient reference lake Black Pond maintained three daphniid species throughout the ca. 150-year record. Species richness increased in the three eutrophying lakes, with two species gained in Roseland and Cedar and one gained in Alexander over the historic record. Similar patterns were recorded using rarefied species richness estimates (ESM4: figure S5).

## Discussion

Land use change resulting in habitat loss and modification is often associated with biodiversity loss and homogenisation of the world's biota [2,4]. Here we demonstrate that nutrient and heavy

metal pollution, two widespread forms of chemical pollution associated with anthropogenic activities, explained key changes in the composition of daphniid zooplankton assemblages over the past ~140 years. However, contrary to expectations, these responses resulted in an overall increase in species richness in eutrophying lakes owing to colonisation by large-bodied daphniid taxa. While we observed some evidence of homogenisation of daphniid assemblages in three eutrophying lakes, this pattern depended on the similarity measure used, and patterns of divergence from the low-nutrient reference site were inconsistent. Overall, eutrophication was the dominant force explaining historical changes in community structure in fertilised lakes (table 1, figure 2 *b-d*). Contamination by metals alone explained subtle shifts in species relative abundances in the oligotrophic reference lake. While caution is warranted when extrapolating based on patterns observed in four study lakes, the pervasive nature of lake eutrophication [12,46], and metal contamination [22], combined with the sensitivity of cladocera to both factors [15,32], suggest that daphniid zooplankton assemblages in other lake regions with ubiquitous agricultural or urban development may have followed similar trajectories [12].

Although daphniid species composition and relative abundances responded to both eutrophication and metal contamination in study lakes, effects of nutrients were paramount in the nutrient-rich sites, both uniquely and in combination with metal contamination (table 1). We observed colonisation by *D. mendotae* and *D. pulicaria* in eutrophying lakes and shifts in relative abundance of other taxa (figures 1, 2 and 4), but no extirpation of native species. Elevated phytoplankton abundance due to eutrophication is expected to favour large-bodied *Daphnia* such as *D. pulicaria* and *D. mendotae* [30], consistent with their absence from the oligotrophic reference lake and low densities prior to eutrophication. This species sorting from the regional species pool fostered a gain of taxonomic diversity, both in terms of species richness per site



(alpha) and the total number of species found across sites (gamma). Further, our estimates of changes in historical diversity may represent a minimum value, as we did not correct for increased sediment accumulation rates and dilution of subfossils which can occur in both modern sediments and with eutrophication [56].

Temporal patterns of *D. mendotae* and *D. pulicaria* colonisation and population fluctuations appeared to exhibit a threshold response to fertilisation. Although colonisation of these species occurred early in the sedimentary record of eutrophying lakes, substantial increases in relative abundances of these taxa were restricted to the past 10-30 years, after eutrophication had progressed substantially (figure 1). Large-bodied *D. mendotae* and *D. pulicaria* may require high algal abundance to achieve stable dominance [57,58]. Interestingly, colonisation and establishment of *D. galeata*, a species closely related to *D. mendotae*, also appears to require a eutrophication threshold in European lakes [33].

Spatial dispersal of *Daphnia* resting eggs undoubtedly enabled the colonisation of new species in the eutrophying lakes; however, it seems unlikely that an increase in dispersal alone would explain the recent rapid increase in *D. mendotae* and *D. pulicaria*. Owing to their large body size, increased dispersal opportunities might make colonisation by these species more likely, but other smaller taxa in our assemblages should disperse even more frequently. In a study of colonisation patterns of Midwestern USA reservoirs, *D. pulicaria* and other large-bodied cladocerans were also slower to colonise [59]. Allen *et al.* attributed this pattern to reduced dispersal ability in larger-bodied cladocerans [59]; however, it is also likely that the increasing eutrophic status of these reservoirs favoured establishment of these large species.

In eutrophying Cedar Pond, elevated Cu concentrations were associated with the disappearance of *D. pulicaria* for several decades; this species reappeared and flourished when

Cu levels approached baseline levels (figures 1, 2c). As densities of *D. pulicaria* were near the detection limit throughout much of the record in Cedar, it is possible that this species was not absent but rather extremely rare in the 1960s-90s. The rapid rise in Cu in Cedar in the 1950s-1980s, particularly separately from other metals, is consistent with the idea that CuSO<sub>4</sub> algicide was applied to Cedar Pond during this period of *D. pulicaria*'s absence. Similarly, we hypothesize that application of CuSO<sub>4</sub> may have prevented *D. pulicaria* from establishing and flourishing in eutrophying Roseland Lake.

Interestingly, with the exception of copper in Cedar Pond, pollution with metals alone had no measurable unique effect on daphniids in fertilised lakes, even though the magnitude of change in metal influx was apparently similar to or greater than that of nutrients associated with eutrophication, and several metals reached potentially-toxic levels (ESM4: figure S2). Effects of metal contamination alone resulted in subtle shifts in community structure only in oligotrophic Black Pond. Overall, *Ceriodaphnia* was strongly negatively associated with metal increases (figure 2) and has declined in all lakes where present (figure 1). This is consistent with laboratory findings that *Ceriodaphnia* usually exhibits high sensitivity to chemicals during *in vivo* toxicity trials [32]. One possible explanation for the relative lack of importance of metals is that rapid evolutionary responses of the *Daphnia* assemblages have obscured ecological impacts of metals. However, an empirical study of *Daphnia* populations in these lakes actually found evidence of maladaptation to metal contamination [60].

Despite the paramount effect of nutrients, variance partitioning analysis suggested that daphniid community composition was sensitive to the combined effects of fertilisation and metal pollution (table 1). This could occur because synchronous changes in metal contamination and eutrophication make it impossible to statistically tease apart their relative influences. However, it

is also possible that metals and eutrophication have a synergistic effect on daphniid species patterns. Such a synergy could arise because eutrophication favours lower oxygen content in deep water, thereby elevating metal release from sediments [61]. Consistent with this interpretation, we note that the carotenoid okenone from obligately-anaerobic purple sulphur bacteria was more common in both Cedar Pond and Alexander Lake after several decades of fertilisation, indicating repeated periods of anoxia in these lakes. In addition, fertilisation was accompanied by other changes in water chemistry that may have influenced metal toxicity [46]. For example, laboratory analyses show that daphniid metal sensitivity increases when accompanied by low concentrations of calcium [62], an element which has declined in Alexander Lake, Roseland Lake and Black Pond after ca. 1970 [46]. Although further research is needed to differentiate among these mechanisms, together our findings suggest that complex, hierarchical interactions between eutrophication and metal pollution may control daphniid community composition.

Colonisation by *D. pulicaria* and *D. mendotae* led to an increase in Jaccard similarity in the three eutrophying lakes; however, this consistent homogenisation pattern was not observed in the multivariate ordination of the daphniid assemblages (figure 3a). Shifts in daphniid composition in the eutrophying lakes followed similar trajectories along PC axis 3 (figure 4). However, the Euclidian distance, which incorporates variation along the first three PC axes (figures 4 and S4), showed that eutrophying Alexander and Roseland became more similar, while both diverged from Cedar (figure 3a). Daphniid assemblages in eutrophying Alexander Lake and Cedar Pond diverged from the assemblage in the unproductive reference site, Black Pond (figure 3b); however, daphniids in Roseland became more similar to those in Black Pond. Although speculative, we suggest the annual application of  $\text{CuSO}_4$  to control primary

productivity in Roseland caused the daphniid assemblage to resemble that of the low-nutrient lake, an undesirable effect as large-bodied *Daphnia* species are effective biological controls of phytoplankton production [63].

This study focused on daphniid zooplankton because they are keystone organisms in freshwater food webs [29], are well preserved in sediments [41], can be identified to species by hatching and culturing diapausing embryos, and are known to be sensitive to both nutrient and metal pollution [15,30–32]. However, many other zooplankton species inhabit these lakes, and it remains unknown how they may have responded to eutrophication and metal contamination. Similarly, it is difficult to extrapolate from four lakes, including a single reference lake, to a broader regional trend. However, we note that surveys of regional lakes have demonstrated a decline in transparency and an increase in nutrient concentration since 1930 [46]. Furthermore, a 2011 survey of 14 Connecticut lakes shows that *D. mendotae* and *D. pulicaria*, are usually absent from oligotrophic lakes in this lake district (ESM5: text S5, table S5, figure S6). Thus, while we have limited knowledge of historic regional daphniid compositional changes, the frequent occurrence of these taxa associated with elevated nutrients, combined with the fact that many lakes have eutrophied, support the idea that the shifts in daphniid composition observed in our study lakes could be more widespread.

Chemical alteration of the environment by addition of nutrients and metals played a significant role in explaining the patterns of daphniid zooplankton assemblage shifts documented here. Eutrophication of lakes is ubiquitous in inhabited regions [12], while contamination with metals affects lakes in both industrial landscapes [22] and more remote sites [25]. In addition, application of  $\text{CuSO}_4$  algacide is a widespread management practice that still occurs in many lakes in the US today, despite its high toxicity to aquatic life and limited effectiveness in

regulating symptoms of eutrophication [64]. In regions of the world where significant land conversion and/or industrial activity began relatively early (e.g. Europe, Asia), anthropogenic eutrophication and metal contamination have been impacting lake ecosystems for centuries (e.g., [65–67]). Given these observations, modest extrapolation of our findings suggests that eutrophication and metal contamination may have driven long-term shifts in daphniid zooplankton assemblages in inhabited catchments around the world. Whether such community responses have occurred and how they might affect the functioning of *Daphnia* populations in these lakes are questions that deserve further attention.

**Data accessibility** Supporting data have been uploaded to Dryad (doi:10.5061/dryad.2vh5c) in keeping with the journal’s policies.

**Competing interests** We have no competing interests.

**Author contributions** MAR and DKS contributed to the conception and design of this study. MAR and PRL contributed to data acquisition, and MAR, DKS, and PRL contributed data analysis and interpretation. MAR drafted the manuscript and all authors contributed to revisions. MAR, DKS, and PRL agreed on the final version to be published and are accountable for the accuracy and integrity of the work.

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**Figure legends:**

**Figure 1.** Changes in daphniid taxa relative abundances, heavy metal contamination, and eutrophication in the four study lakes over the past 125 years+. Relative abundances of daphniid taxa over time in the four lakes are based on analysis of diapausing egg banks. Metals and eutrophication data represent the first PC scores for analyses conducted for each lake. ESM4: table S3 and figures S1-S2 provide additional details and plots of raw data.

**Figure 2.** RDA triplots showing significant ( $\alpha < 0.05$ ) relationships between eutrophication (E) or metal (M) PC1 scores, copper (Cu), in the case of Cedar Pond, and Hellinger transformed daphniid species densities. High values for M and E indicate higher concentrations of metals and greater eutrophication respectively. Since only one explanatory variable was significant in the RDAs of Black, Alexander, and Roseland, it is only possible to display one RDA axis for these plots. The X axes (RDA 1) show the relationship between eutrophication or metals and daphniid composition, while the Y axes (PC 1) show unconstrained (residual) variation. Species scores are represented by abbreviated species names: *Ceriodaphnia* = CER, *D. ambigua* = AMB, *D. catawba* = CAT, *D. mendotae* = MEN, *D. parvula* = PAR, *D. pulicaria* = PUL. Years indicate daphniid composition (site scores) at each time period.

**Figure 3.** Changes in similarity between modern and historic daphniid assemblages in the study lakes. Community similarity measured with PCA is based on 3D Euclidean distances using PC1-PC3 axis scores. For both Jaccard similarity ( $J$ ) and PCA distance, increasing values indicate greater similarity in modern sediments. Decreasing (negative) distances indicate divergence over time. Panel *a* compares assemblages in eutrophying lakes; panel *b* compares

eutrophying lakes with the low-nutrient reference site, Black Pond. Lake abbreviations: B: Black, A: Alexander, C: Cedar, R: Roseland. Modern *J* and PCA distances compare assemblages in surface sediment (ca. 2011). Historic *J* and PCA distances compare assemblages ca. 145-115 years before present, matching sediment ages in each lake pair as closely as possible. Years used in historic comparisons include: B-C 1873-1863, R-B 1863-1873, A-B 1888-1899, A-C 1888-1898, A-R 1888-1863, R-C 1863-1863.

**Figure 4.** PCA biplot showing temporal shifts in daphniid assemblage composition in each lake. The PCA is based on Hellinger transformed species densities estimated from sediment diapausing egg banks. PC1 and PC3 are plotted to show as much variation in daphniid community structure as possible, while ensuring that all six taxa were important in explaining at least one axis of the plot. Biplots of PC axes 1-2 and 2-3 are provided in ESM4 figure S4. ESM4 table S4 provides additional PCA results. Species scores are represented by abbreviated species names: *Ceriodaphnia* = CER, *D. ambigua* = AMB, *D. catawba* = CAT, *D. mendotae* = MEN, *D. parvula* = PAR, *D. pulicaria* = PUL. Site scores are labelled with the approximate age of the sediment for that time period. Lines are drawn to show the temporal changes in daphniid composition within each lake. Alexander=blue, Cedar=gold, Roseland=red, Black=black.

**Tables:**

**Table 1.** Variance partitioning of RDA-models relating daphniid assemblage structure (Hellinger transformed species abundances at various time periods) with metal contamination (PC1) and eutrophication (PC1) (electronic supplementary material, table S2). Separate analyses were conducted for each lake. Amount of variation explained solely by eutrophication and metals as well as their overlap is displayed. Overlap between the effects of nutrients and metals cannot be statistically distinguished, as this variation could be explained by two independent mechanisms running in parallel.

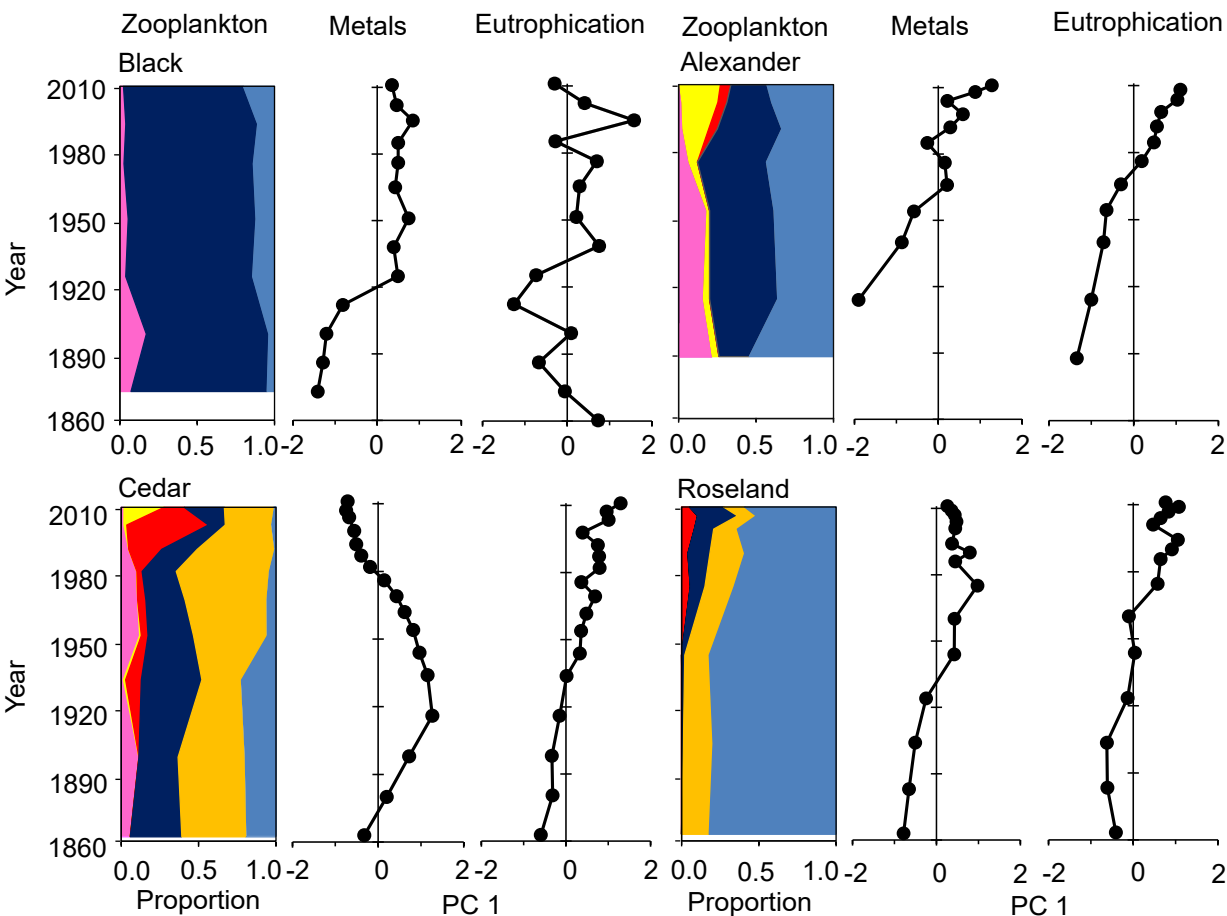
Lake	Index	Adj R <sup>2</sup>	F	P value
Black	Nutrients	0.000	1.00	0.416
	Nutrients + Metals	-0.185 <sup>a</sup>	--	--
	Metals	0.716	8.64	0.039
Alexander	Nutrients	0.254	5.10	0.064
	Nutrients + Metals	0.536	--	--
	Metals	-0.038	0.38	0.844
Cedar	Nutrients	0.346	4.636	0.017
	Nutrients + Metals	0.135	--	--
	Metals	-0.069	0.280	0.855
	Nutrients + Copper	-0.160	--	--
	Metals + Copper	-0.010	--	--
	Nutrients + Metals + Copper	0.039	--	--
	Copper	0.148	2.556	0.062
Roseland	Nutrients	0.391	10.54	0.011



Nutrients + Metals	0.380	--	--
Metals	-0.180	0.570	0.561

658   <sup>a</sup> Note that non-significant terms can have a negative adjusted  $R^2$ , which can affect the adjusted  
659    $R^2$  of the significant terms.

660



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*Ceriodaphnia* *D. ambigua* *D. catawba* *D. mendotae* *D. parvula* *D. pulicaria*

